Nanoparticles with Immobilized Biosensors for Bioactive Papers

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Imagine a Bioactive Paper Towel

- That would change color to tell us when our kitchen counter top was contaminated with dangerous bacteria.
- That would change color to warn us of the presence of anthrax spores.
Imagine a Bioactive Mask

Which will:

- *warn* the user of viral contamination.
- *capture* and *deactivate* the virus.
Imagine a Bioactive Water Filter

- Specifically bind small, soluble (i.e. difficult to remove) toxins
- Capture and kill all bacteria and virus.
- Warn user of contamination and when the cartridge must be changed.
a Network of Canadian Academics
~ 28 professors, 11 Universities, ~ 70 total students, PDFs and profs.
~ $2.5 million / year
  o 75% from Canadian Government’s Natural Science and Engineering Research Council (NSERC Network)
  o 25% cash from industrial consortium and Ontario Government.
Bioactive Paper - inexpensive paper products which detect and repel or deactivate waterborne and airborne pathogens.
Key Elements of **SENTINEL** Vision

- Paper giving **instant** visible indication of pathogens.
- **High speed** manufacture – coating or printing.
Bioactive Paper – a “Top” idea

- Described the Sentinel Bioactive paper concept as one of the top 70 in “The 7th Annual Year in Ideas”
“detect” pathogens

♦ Instant, visible indication of pathogens without instrumentation
♦ Technologies do not exist – this is the "killer application" for bioactive paper
♦ A large part of the SENTINEL research
Agents which specifically recognize (capture) a target

Sentinel researchers are developing four technologies in parallel

- Antibodies
- Bacteriophage
- DNA aptamers
- Enzymes
Applying Biodetection Agent to Paper

- To fibers before papermaking process
- Coating
- Printing on paper
Approach 1 – Simply Print a Solution

- Case 1 – no adhesion to paper surface
  - Example: DNA aptamers on cellulose
  - Exposure to target or developing solutions causes biodetection polymer to come off paper

- Case 2 – very strong adhesion with surface
  - Example: any anionic protein on wet strength resin coated paper
  - Enzymes and antibodies may denature on surface
Approach 2 – Cellulose Binding Domains

- Cellulase – family of enzymes which degrade cellulose
- Two parts – binding domain, catalytic domain (CBD)
- Sentinel researchers have genetically engineered CBD onto antibodies and phage
- Gives spontaneous binding to pure cellulose with controlled orientation
- What about surfaces coated with size, wet strength resin, retention aids, and/or fillers?
Approach 3 – Carrier Particles

- Attach biodetection molecules to carrier particles which can be formulated into ink
- The particles isolate the detectors from the paper surface chemistry
- Three Sentinel initiatives:
  - Porous silica particle *Brook/Brennan*
  - Microcapsules *Rochefort/Paice*
  - Microgels *Pelton/Filipe/Li/Hall*
Microgel Carrier Particles
Microgel Preparation

- Essentially a surfactant-free emulsion polymerization
  - Particles form because polymer is insoluble at high temperature
  - Electrostatically stabilized – sulfate from KPS.
  - Surfactant will give smaller particles

\[
\text{BA} \quad \rightarrow \quad \text{NIPAM}
\]

Water, 70°C

PNIPAM microgel
All Microgel Properties Depend Upon T

Below 33°C
- **Microgels**
- about 90% water
- transparent

Above 33°C
- latex particle
- about 20% water
- white
Swollen gel on TEM grid

Dehydrated Gel on TEM grid - cross section

Pelton and Chibante, Colloids and Surfaces 20, 247 (1986)
Microgels for Bioconjugation

- Require functional groups
- We chose carboxyl groups
- The pioneers:
  - Kawaguchi (1992) - first microgel protein interactions
  - Pichot & Elaissari - microgel with grafted oligo-DNA
Microgel Derivatization

- IgG or Aptamer still functions in solution after coupled onto microgel surface.
Microgel Swelling

Hydrodynamic Diameter (nm)

pH

APT-MG
SP-MG
IgG-MG
MG
RB-MG

~ 0.2 mg/m² streptavidin on microgel surface
Electrophoretic Mobility

SP-MG
APT-MG
RB-MG
IgG-MG
MG
DNA Aptamer Detecting ATP


Synthetic short DNA chains which fold to capture a specific target.
Schemes of the Set-up for Detection

Scheme 1
- Elution
- Filter paper
- Microgel
- Sample to be detected

Scheme 2
- Microgel
- Sample solution
“Spotted” Microgels on Filter Paper

- Microgel penetrates into filter paper about 1/3 of the thickness.
- Microgels do not move with elution
Inkjet Microgel Printing

- Dimatix Fujifilm Printer
Printing IgG-MG or APT-MG onto Filter Paper Surface

* IgG or Aptamer coupled on microgel can survive the printing process.
* Promising for making microgel-based bioinks.

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Cationic Paper

- DNA aptamer is denatured when directly applied to cationic paper.
- Microgel supported aptamer functions on cationic paper.

<table>
<thead>
<tr>
<th>Aptamer Directly Applied to PAE Treated Paper</th>
<th>APT-MG on PAE Treated Paper</th>
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<tr>
<td>ATP  GTP</td>
<td>ATP  GTP</td>
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Aptamer

Microgel supported aptamer functions on cationic paper.
Summary

- Microgels are immobilized on filter paper
- Microgel supported antibodies and DNA aptamers retain activity on filter paper
- Microgel protects aptamer from cationic polymer impregnated wet strength paper
That’s All

Bioactivepaper.com
Papersci.mcmaster.ca
Microgel Superstar

- B.Eng, Queens
- Ph.D. McMaster 2006
- MIT PDF 2006-2008
- New Faculty at McMaster July 2008

Todd Hoare
**Microgel Microstructure Models**

![Graphs showing COOH concentration profiles for MAA-NIPAM, AA-NIPAM, and VAA-NIPAM models.](image)

- **MAA-NIPAM (Inverse Core-Shell Model)**
  - \( r_{\text{NIPAM}} = 0.20 \pm 0.08 \)
  - \( r_{\text{MAA}} = 2.8 \pm 0.4 \)

- **AA-NIPAM (Core-Shell Model)**
  - \( r_{\text{NIPAM}} = 0.57 \pm 0.07 \)
  - \( r_{\text{AA}} = 0.32 \pm 0.04 \)

- **VAA-NIPAM (Surface Model)**
  - \( r_{\text{NIPAM}} = 16.7 \)
  - \( r_{\text{VAA}} = 0.002 \approx 0 \)

Direct correlation between monomer reaction kinetics and resulting COOH distributions.

Chain Transfer with Vinylacetic Acid

- Slow propagation kinetics and chain transfer ability of VAA
  → expect chain end, surface-localization of functional groups
  A “one-step” method of making functionalized “hairy” microgels?
Detection of Specific Target by APT-MG (Scheme 2)

- APT-MG was mixed with the quencher before applying.
- Paper strips were eluted in ATP or GTP solution.
- Elution in ATP, specific target, enhanced the fluorescence intensity.
PAE Structure and Reactions

- Cationic, reactive water soluble polymer
- Reacts with carboxyl and amine groups
- Requires heat to drive reactions
- Produces positively charged paper

Microgel Swelling

- Gel swelling decreases at LCST.
- \( \sim 10 \) times decrease in volume
- Swelling sensitive to ionic strength
DNA Aptamer with Built-in Reporting

Structure-switching signaling

FDNA
FȘTCACTGACCTGGGGGGGAGTATTGCGGAGGAAGGT
Q-GTGACTGGGACCC 5'
QDNA

Target

Non-target

Protein or DNA oligos will Pass Through the Microgel on Paper Surface

- Proteins have molecular level contact with microgel
Detection of Antigen by IgG-MG (Scheme 2)

A: (1.6 µg/ml Ag-Per or Per)

B: (0.16 µg/ml Ag-Per or Per)
Detection of Antigen by IgG-MG (Scheme 1)

- Antigen was conjugated with peroxidase.
- Further elution in OPD, substrate for peroxidase, gave out color signals.
- Only the strip with antigen showed brown color